Development of a formulated diet for mud crab, *Scylla serrata,* larvae

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STATEMENT ON SOURCES

DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

JEROME GENODEPA

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ABSTRACT

This study aimed to develop a suitable formulated diet to replace live food in mud crab, *Scylla serrata*, larval culture. A microbound diet (MBD) was formulated based on known requirements of other crustaceans and was assessed for ingestion and retention by the various larval stages of *S. serrata.* Ingestion and retention was determined by labeling the MBD with ^{14}C, and subsequently measuring ^{14}C in larvae fed these diets. After it was shown that the larvae readily accepted MBD, different types of binders were tested to determine those best suited for MBD prepared for *S. serrata* larvae. The diet particle size preference and optimal feeding ration were then determined for the various larval stages. Finally, the diet was tested in combination with different ratios of *Artemia* as a food source for Megalopa.

Fundamental to the success of the study was the development of methods that would ensure routine and reliable production of mud crab larvae. Larval production runs were tried using protocols from various authors and procedures that showed positive results after several trials were adopted. A progressive improvement in survival was achieved towards the end of the study and the rearing protocol that was finally adopted has now formed the basis for hatchery production of *S. serrata* at James Cook University.

A technique for evaluating fish larvae using dietary ^{14}C was refined and adopted in this study for the measurement of ingestion and retention of the MBD by *S. serrata* larvae. Several studies were conducted to serve as basis to refining this technique. Based on an experiment to determine factors that could affect the measurement of the ^{14}C content of

larvae fed ^{14}C labelled MBD, it was found that *S. serrata* larvae do not absorb the ^{14}C that leaches from the diet but MBD particles that stick to the larvae were the major source of potential error. As such, it was found necessary to include a control treatment with dead larvae when running an experiment so that the radioactivity reading of the dead larvae can be used to correct the radioactivity readings in the treatments when measuring ingestion.

Newly hatched *S. serrata* zoea readily ingested the MBD and ingestion increases with larval age. Ingestion of MBD did not vary significantly between Zoea I and Zoea II and also between Zoea III and Zoea IV; ingestion by other larval stages were significantly different from each other. Ingestion of MBD by Megalopa was found comparable to previously reported ingestion of live food (8 *Artemia* larva^{-1} h^{-1}).

Studies were also conducted to determine the duration of diet exposure that resulted in maximal ingestion of the MBD. Results showed that for Zoea I to Zoea III and Megalopa, feeding for 1 h resulted in maximal ingestion as there was no further increase in ingestion with longer diet exposure. For Zoea IV and Zoea V, at least 2 h was required for maximal ingestion as there was no further increase in ingestion after 2 h.

The gut residence times (GRT) of MBD for the various larval stages of *S. serrata* were determined in order to know the required period that should be allowed for the larvae to empty their gut when measuring retention of the MBD. In Zoea I, GRT was found to be 1 h as retention of the MBD significantly decreased after 1 h following removal of

available MBD. In Zoea III and Zoea IV, GRT was found to be 2 h; retention of the MBD significantly decreased after 2 h following food removal and there was no further significant decrease in retention after this time. Results did not clearly indicate the GRT of Zoea V and Megalopa, but there were indications suggesting longer GRT of around 4-5 h for these stages.

Studies to test the suitability of different binders (agar, alginate, carrageenan, gelatin and zein) for MBD showed that there were no significant differences in ingestion and retention of MBD resulting from binder type. Further evaluation of these binders, based on leaching of radioactivity from diets, showed that least leaching was found in zein-bound MBD. Since the greater leaching of nutrients from diets with other types of binders did not make these diets more attractive (i.e. result in significantly greater rates of ingestion), the minimal leaching of nutrients from zein-bound MBD made zein the more desirable binder. Unnecessary leaching wastes important dietary components and can result in deterioration of water quality.

The particle size preference and optimal feed ration were determined for the various larval stages of *S. serrata* based on larval ingestion of ^{14}C labeled MBD. The results provided important information for feeding management of *S. serrata* and allowed recommendation of the most suitable MBD particle size range and ration for each of the larval stages of *S. serrata* (i.e. for Zoea I, <150 µm MBD particles given at 5.4 mg L^{-1}; for Zoae III, 150-250 µm MBD particles fed at 7.1 mg L^{-1}; for Zoea V, 250-400 µm MBD particles fed at 8.2 mg L^{-1}; for Megalopa, 400-600 µm MBD particles given at 2

mg L^{-1}). It was found that the optimal particle size ranges for different larval stages are not completely provided by a rotifer/*Artemia* diet commonly used in mud crab hatcheries. This highlights the advantage of using MBD since they can be prepared within any desired particle size range and as such offer the potential to provide a more appropriate diet to *S. serrata* larvae. The results also suggested that MBD, provided at a rate equivalent to 50% of the dry weight of the 'standard' live food diet is the optimal ration for Zoea I to Zoea V larvae and it could be as low as 12.5 % for Megalopa.

The potential for complete and partial replacement of *Artemia* with MBD for Megalopa was also tested. Survival of megalopae to crab stage did not vary significantly between the different ratios of MBD and *Artemia*, but a combination of 25 % MBD and 75% *Artemia* consistently gave the highest survival. Treatments receiving high proportions of MBD molted earlier compared to those receiving high proportions of *Artemia*. In another experiment where Megalopa were reared individually, 90 % survival to crab stage was achieved in both treatments fed either MBD or *Artemia* only. The megalopae fed MBD only also molted one day earlier than those fed *Artemia* only. These results showed that the MBD was capable of supporting successful molting of megalopae to crab stage and the possibility of complete replacement of *Artemia* with MBD. It was also shown that while both the MBD and the *Artemia* were adequate feeds on their own, a combination of the two in an appropriate proportion may give improved results.